LEUKOPENIA AND LEUKOCYTOSIS IN RABBITS*

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In view of the renewed interest in therapeutic injections of dead bacteria, a study of the resulting changes in the leukocytes is pertinent. There regularly occurs in the blood, following intravenous injection of foreign proteins, 2 phenomena: first, within a few minutes, a marked reduction of leukocytes, a condition to which Löwit[1] has applied the term leukocytopenia or leukopenia; and second, after several hours, a marked increase of leukocytes, a condition termed hyperleukocytosis. My paper deals with these phenomena as they occur in the blood of rabbits after the injection of certain bacteria.

HISTORICAL REVIEW

The recognition of a condition of leukopenia in the blood by Wyssokowitsch in 1886, and later by Löwit in 1892, gave rise to 2 general theories in explanation thereof: (1) that the diminution of leukocytes is due to an actual destruction of the cells (Löwit), and (2) that it is due to a withdrawal of the leukocytes from the circulating blood into the capillaries of various organs (Goldscheider and Jacobs).[2] Löwit was led to his conclusion through failure to examine the blood of the various organs and through finding of a few degenerated white blood cells in the circulating blood. His theory received the support of Römer. Goldscheider and Jacobs, who had produced a leukopenia by intravenous injection of spleen, thymus, and bone-marrow extracts, concluded that the diminution of leukocytes was due to a retention of these cells in the capillaries of the lungs, spleen, and liver as the result of a negative chemotactic influence on the leukocytes by the injected substances. Silverman[3] considered the withdrawal of the leukocytes from the circulating blood to the capillaries to be the result of a mechanical obstruction in the capillaries caused by swelling of the endothelial cells, this obstruction sifting out the leukocytes and holding them within the organs. Ewing,[5] however, claimed to have found a similar swollen condition of endothelial cells in sections of normal livers. Bruce[4] counted the number of leukocytes in a given field in microscopic sections of lung, spleen, and liver, both in normal animals and in tissues taken during leukopenia, and found in the latter condition a great increase of leukocytes. Ewing[5] found an average of 17,400 leukocytes per c.mm. in sections of normal liver tissue, the blood from the ear at the corresponding time containing 8500 leukocytes, while in a section of liver taken during leukopenia, he counted 40,000 leuko-

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[1] Studien z. Physiol. u. Pathol. d. Blutes v. d. Lymphe, 1892.

[2] Ztschr. f. klin. Med., 1894, 25, p. 373.

[3] Univ. of Penn. Med. Bull., 1904-05, 17, p. 22 (contains a complete review of the literature).

[5] New York Med. Jour., 1895, 61, p. 257.

[4] Proc. Roy. Soc., 1894, 55, p. 295.

cytes per c.mm., the blood from the ear vein containing only 3500. These observers and others asserted that the reduction of the leukocytes was due to accumulation of these cells in capillaries of the internal organs, especially those of the lungs, spleen, and liver, and not to disintegration.

The early observations on the effect of protein-injection recorded only the hyperleukocytosis, overlooking entirely the preceding leukopenia. Thus Hirt in 1856 noted an increase in leukocytes following injection of tincture of myrrh; Binz in 1876 injecting ethereal oils, and Pohl injecting numerous other drugs, obtained similar results. Several theories arose to explain the phenomenon. Limbeck's exudative theory assumed leukocytosis to be dependent on the existence of a localized exudation at the point of infection, the larger the exudate the greater the leukocytosis. Löwit's[1] theory assumed that all conditions of leukocytosis are contingent on a preceding destruction of leukocytes, the latter serving to stimulate the formation of new leukocytes. Schulz and Rieder,[6] failing to find any actual increase of leukocytes during any stage of leukocytosis, explained the phenomenon as a difference in distribution, the leukocytes in hyperleukocytosis being merely withdrawn from the internal organs to the peripheral vessels. Pfeiffer applied the principle of chemotaxis to the production of leukocytosis, and all the later theories are founded on this principle. Ehrlich[7] believed the leukocytosis to be due to an attractive chemotaxis exerted by microorganisms or toxins on a large reserve of neutrophiles always present in the bone marrow. The most generally accepted explanation of leukocytosis is that formulated by Goldscheider and Jacobs,[2] which explains leukopenia and leukocytosis on the basis of a repellant and an attractive chemotaxis, the first causing a retreat of the leukocytes into the capillaries of the internal organs, the second causing an outpouring of these leukocytes, as well as of those newly formed in the bone marrow, into the general circulation. No explanation, however, is advanced for the change from the repellant, to the attractive chemotactic phase.

TECHNIC

Rabbits were selected for this study on account of the ease with which they may be handled and the relative stability of their leukocytes under ordinary conditions. Three series of animals were studied, including 6 apparently normal rabbits, 9 in which examinations of the leukocytes were made during leukopenia, and 6 in which examinations were made during hyperleukocytosis. In every rabbit counts of the leukocytes, both numerical and differential, were made of blood from as many sources as possible, including that which oozed from the cut surface of the parenchyma of the spleen, liver, lung, and bone marrow. In the differential counts at least 100 cells were counted. The blood smears were stained 1 minute in a 1% methyl alcoholic eosin solution, followed by one-half minute in 1% methyl alcoholic methylene-blue solution. All the rabbits were kept without food for 12 hours preceding the injection and during the entire experiment. Ether was the anesthetic, the animals being restrained as little as possible. The bacteria, consisting of typhoid bacilli, streptococci, and staphylococci killed by heating to 60 C., were injected into the ear vein; the quantity injected varied, that of typhoid bacilli from 3½ to 60 million, streptococci from 2 to 60 million, and staphylococci from 20 to 60 million organisms. In the case of several rabbits emulsions of living streptococci were injected. Small pieces of spleen, liver, lung, and bone marrow were taken during each stage, and fixed in saturated alcoholic solution of corrosive sublimate.

[6] Beitrage zur Kennt. d. Leucocytose, 1892.

[7] Die Anaemie, in Nothnagel's Specielle Path. u. Therapie, 1898.

THE LEUKOCYTES IN NORMAL RABBIT BLOOD

The number of leukocytes in the blood obtained from the general circulation varied in different normal rabbits (Table 1). The number per c.mm. from the ear vein varied from 3300 to 14,650, averaging about 9000. A rather constant finding was the lower count for blood from the left ventricle, as compared with that from the right — a condition probably due to retention of leukocytes in the lung capillaries, either as a normal process or as the result of irritation from the anesthetic. The counts of blood from the liver and lung parenchymata approximated those for the general circulation; the counts of blood from the splenic parenchyma averaged about 3 times those of the circulating blood.

Table 2 presents typical numeric and differential leukocytic counts of blood from various sources in a normal adult rabbit. In most instances a slight increase of leukocytes was found in blood from the veins of the principal organs; in blood from the splenic parenchyma the count was several times that of the peripheral blood, a fact to be explained perhaps by the function and structure of this organ. Care was exercised to avoid pressure on the spleen and other organs while blood was being obtained from the parenchyma. Usually the blood from other sources contained a fairly uniform number of leukocytes. The blood of a normal rabbit contained 20-30% polymorphonuclear cells, 55-74% small lymphocytes, 4-15% large lymphocytes, and 3-10% mast cells.

THE LEUKOCYTES IN LEUKOPENIA

Nine adult rabbits were selected for the study of leukocytes during the leukopenia produced by intravenous injections of bacteria. The quantity injected varied in different rabbits from the smallest dose of 3½ million dead typhoid bacilli to the largest of 60 million dead typhoid bacilli, streptococci, or staphylococci (Table 3).

The onset of leukopenia occurred in most cases within 10 minutes after injection. In Rabbit 33 there was a drop from 5100 to 2200 leukocytes per c.mm. in the peripheral blood within 2 minutes after the injection of 60 million dead typhoid bacilli; the polymorphonuclear elements during this time decreased from 24% to 1%. In Rabbits 22, 4, and 31 the fall in number of leukocytes per c.mm. in the blood of the ear vein amounted to 8250, 11,450, and 9550, respectively. The lowest count observed during this period was 1250 in the blood of the ear vein of Rabbit 22, the original count being 9500.

TABLE 1

LEUKOCYTIC COUNTS OF BLOOD FROM DIFFERENT SOURCES IN NORMAL RABBITS

Rabbit	Ear Vein	Bone Marrow	Splenic Pulp	Splenic Artery	Splenic Vein	Portal Vein
7	3700	3500	39350	.....	.....	.....
8	8350	16250	25350	.....	.....	.....
23	14650	10350	33500	6350	9400	9100
24	13250	.....	32850	.....	10650	10300
25	8100	11750	62800	11600	16400	6200
30	10900	.....	41500	15300	.....	.....

TABLE 2

TOTAL AND DIFFERENTIAL COUNTS OF LEUKOCYTES IN BLOOD FROM VARIOUS SOURCES IN THE ADULT NORMAL RABBIT *

Source of Blood	Total Count	Differential Count							
		Polymorphonuclears		Small Lymphocytes		Large Lymphocytes		Mast Cells	
		No.	%	No.	%	No.	%	No.	%
Ear vein	14650	2628	18	9636	66	1314	9	1022	7
Bone marrow	10350	1449	14	6624	64	1139	11	1138	11
Splenic vein	9400	2726	29	5170	55	940	10	564	6
Splenic pulp	33500	5025	15	22445	67	5025	15	1005	3
Splenic artery	6350	1270	20	4445	70	381	6	254	4
Superior mesenteric vein	6350	1588	25	4127	65	317	5	318	5
Superior mesenteric artery	6000	780	13	4440	74	360	6	420	7
Liver pulp	5250	1365	26	3308	63	262	5	315	6
Right ventricle	7150	1073	15	5219	73	286	4	572	8
Left ventricle	4950	1188	24	3267	66	248	5	247	5
Hepatic vein	11700	1989	17	7839	67	1404	12	468	4
Portal vein	9100	1001	11	6734	74	1092	12	273	3

* Rabbit 23, Table 1.

TABLE 3

COUNTS OF LEUKOCYTES OF BLOOD FROM VARIOUS SOURCES DURING LEUKOPENIA PRODUCED BY INJECTION OF BACTERIA

Rabbit and Its Bacterial Injection	Before Injection (Ear Vein)	During Leukopenia (from 2 to 20 min. after Injection)				
		Ear Vein	Bone Marrow	Splenic Artery	Splenic Vein	Splenic Pulp
1 (40 million streptococci)	14500	5550	30100	.....	.....	130100
4 (60 million staphylococci)	20400	8950	28100	.....	16650	186400
10 (40 million staphylococci)	18650	11000	26500	.....	.....	184000
22 (3½ million typhoid bacilli)	9500	1250	10050	.....	2600	130900
28 (60 million typhoid bacilli)	13300	6850	.....	8000	5850	69000
29 (60 million typhoid bacilli)	12000	5800	10750	3400	59500	132100
31 (60 million typhoid bacilli)	14550	5000	11600	3750	.....	46400
32 (50 million typhoid bacilli)	8350	5400	30200	5600	6550	74400
33 (60 million typhoid bacilli)	5100	2200	22550	2400	.....	118800

TABLE 1—*Continued*

LEUKOCYTIC COUNTS OF BLOOD FROM DIFFERENT SOURCES IN NORMAL RABBITS

Hepatic Vein	Superior Mesenteric Vein	Superior Mesenteric Artery	Liver Pulp	Right Ventricle	Left Ventricle	Lung Pulp
.....	.....	.....	4000	1600	1150	
.....	.....	.....	4050	3550	1750	
11700	6350	6000	5250	7150	4950	
6950	13900	12200	9950	8950	8900	
2800	5650	4650	6750	3800	1500	4350
.....	.....	.....	1500	13850	6950	10950

Distribution of the Leukocytes.—At the height of leukopenia, the blood in the splenic and mesenteric arteries, splenic, portal, hepatic, and superior mesenteric veins, lung parenchyma, and both ventricles of the heart, usually contained a number of leukocytes equal to that found in the ear vein. The blood of the bone marrow, liver, and splenic parenchyma contained a greatly increased number of leukocytes, especially the blood from the splenic parenchyma, the number from this source reaching 186,400 per c.mm. in Rabbit 4, and over 100,000 per c.mm. in 5 other rabbits (Table 3). In one instance, Rabbit 29, the blood in the splenic vein contained 59,500 leukocytes per c.mm., the blood in the ear vein at the time containing only 5800. In several rabbits the blood in the veins and the left ventricle contained more leukocytes than did that of the corresponding arteries and right ventricle.

Proportions of the Different Types of Leukocytes in Blood from Various Sources.—As a rule there occurred a decrease in the number and proportion of polymorphonuclear leukocytes in the blood from all sources, that from bone marrow and splenic parenchyma excepted, the

TABLE 3—*Continued*

COUNTS OF LEUKOCYTES OF BLOOD FROM VARIOUS SOURCES DURING LEUKOPENIA PRODUCED BY INJECTION OF BACTERIA

During Leukopenia (from 2 to 20 minutes after Injection)

Portal Vein	Hepatic Vein	Superior Vena Cava	Liver Pulp	Superior Mesenteric Vein	Superior Mesenteric Artery	Lung Pulp	Right Ventricle	Left Ventricle
.....	.....	.....	28300					
.....	.....	.....	13650					
.....	.....	.....	24350	.....	.....	.....	11700	12300
.....	.....	.....	2800	1500	1750	.....	3700	1850
3500	10000	.....	11400	6950	5700	.....	6250	6650
3150	5550	.....	7800	3250	4250	4650	4550	8100
10200	18100	11850	15900	7050	7250	7100	5950	12500
12800	.....	8700	14450	5050	4050	9100	8450	6950
.....	4100	.....	9550	2600	3100	3800	5300	13000

TABLE 4

TOTAL AND DIFFERENTIAL COUNTS OF LEUKOCYTES IN BLOOD FROM VARIOUS SOURCES DURING LEUKOPENIA PRODUCED BY INTRAVENOUS INJECTION OF BACTERIA *

Source of Blood and Time of Count	Total Count	Differential Count							
		Polymorphonuclears		Small Lymphocytes		Large Lymphocytes		Mast Cells	
		No.	%	No.	%	No.	%	No.	%
Left ear vein 20 minutes before injection of 50 million typhoid bacilli	8350	1085	13	7098	85	83	1	84	1
Left ear vein 5 minutes after injection	5400	756	14	3240	60	324	6	1080	20
10 to 20 minutes after injection: Bone marrow	30250	7260	24	22385	74	302	1	303	1
Splenic artery	5600	56	1	5376	96	112	2	56	1
Splenic pulp	74400	14136	19	53568	72	5952	8	744	1
Splenic vein	6550	197	3	6232	95	131	2	0	0
Superior mesenteric vein	5050	101	2	4646	92	50	1	253	5
Superior mesenteric artery	4050	0	0	3888	96	122	3	40	1
Liver pulp	14450	289	2	13583	94	289	2	289	2
Portal vein	12800	512	4	11648	91	384	3	256	2
Lung pulp	9100	182	2	8736	96	0	0	182	2
Right ventricle	8450	0	0	8112	96	338	4	0	0
Left ventricle	6950	278	4	6464	93	139	2	69	1
Superior vena cava	8700	87	1	8004	92	348	4	261	3

* Rabbit 32, Table 3.

decrease being synchronous with a drop in the total number of leukocytes. In Rabbit 22, within 15 minutes after the injection, the polymorphonuclear leukocytes dropped from 29% to 0%, and in Rabbit 28 from 22% to 1%; in all rabbits the drop was considerable, tho not always so marked as in the cases specified. The number and proportion of polymorphonuclear leukocytes in blood from the bone marrow were dependent on when during the leukopenia the counts were made; if made early, the proportion was about normal; if late, the proportion as well as the number was much increased. The proportion of poly-

TABLE 5

LEUKOCYTIC COUNTS DURING HYPERLEUKOCYTOSIS PRODUCED BY INTRAVENOUS INJECTION OF BACTERIA

Rabbit and Its Bacterial Injection	Before Injection (Ear Vein)	Count During Hyperleukocytosis				
		Ear Vein	Bone Marrow	Splenic Vein	Splenic Artery	Splenic Pulp
9 (60 million staphylococci)	5750	17000	15600	.....	.....	.....
16 (40 million staphylococci)	11000	37450	34000	.....	.....	41850
20 (50 million staphylococci)	8400	14800	7300	.....	.....	25250
27 (50 million streptococci)	7150	16050	23950	17850	.....	.....
34 (60 million typhoid bacilli)	9650	15700	12200	16000	13600	19100
35 (60 million typhoid bacilli)	9000	24000	19600	91600	16750	73000

morphonuclear elements in splenic blood was approximately that found under normal conditions, but with the enormous increase in the total number of leukocytes in this blood there resulted also a marked increase in the total number of polymorphonuclear elements.

The Condition of the Tissues.—Lung: Appeared normal. Microscopically, there were moderate edema of the capillary endothelial cells and moderate congestion, with an apparent increase in the number of leukocytes. A differential count of leukocytes found in a cross-section of a large vein showed 132 polymorphonuclear, to 15 mononuclear cells.

Liver: Somewhat congested. Smears of the liver parenchyma from a rabbit which had received an injection of living streptococci, contained numerous organisms, both intra- and extracellular.

Spleen: In practically every case markedly distended and bluish from congestion, in some instances twice its normal size. Smears of the pulp from a rabbit which had received an injection of living staphylococci contained numerous organisms.

Bone Marrow: In most instances apparently normal, in a few cases hyperemic. Sections from the tibia and femur revealed a slight increase of the myelocytes with a decrease of fat cells. Phagocytic activity on the part of the giant cells appeared normal.

THE LEUKOCYTES DURING HYPERLEUKOCYTOSIS

Several hours after intravenous injection of bacteria there occurred a marked increase in the number of leukocytes in the blood from all sources (Table 5). The exact time of the increase varied; usually within 3 or 4 hours there was a decided increase, but the acme was frequently not reached until after from 10 to 15 hours. The degree of hyperleukocytosis was not dependent on the number of bacteria injected; Rabbit 9 receiving 60 million dead staphylococci intravenously developed a leukocytosis after 20 minutes, while Rabbit 20 receiving 50 million of the same bacteria did not develop leukocytosis until after 9 hours. The increase was less marked in blood from the splenic parenchyma and bone marrow than was observed during leukopenia.

TABLE 5—*Continued*

LEUKOCYTIC COUNTS DURING HYPERLEUKOCYTOSIS PRODUCED BY INTRAVENOUS INJECTION OF BACTERIA

Count During Hyperleukocytosis								
Hepatic Vein	Portal Vein	Liver Pulp	Superior Mesenteric Vein	Superior Mesenteric Artery	Right Ventricle	Left Ventricle	Superior Vena Cava	Lung Parenchyma
.....	.....	16450	.....	.....	15750	3500		
.....	.....	26850	.....	.....	25900	19350		
.....	.....	10250	.....	.....	4750	5400		
12400	24250	.....	13800	17000	19900	24600	.....	20150
8350	11200	15000	12150	13950	9700	8400	13650	16250
12950	16350	23100	19000	16950	23200	18000	.....	30800

Distribution of the Leukocytes.—The increase, involving chiefly the polymorphonuclear leukocytes (Table 6), was rather uniformly distributed throughout the vascular system with the exception of the splenic vein, and the splenic and lung parenchymata, where the increase was much more marked; as a result the proportion of the polymorphonuclear cells was greatly increased. The actual numbers of small and large lymphocytes remained approximately normal, with a resulting drop in their proportions. The mast cells were not increased.

The Condition of the Tissues.—Lung: No changes. Moderately congested, with an increase in the number of leukocytes. A differential count of leukocytes in a large vein showed 79 polymorphonuclear, to 27 mononuclear cells.

Spleen and liver only slightly hyperemic.

Bone Marrow: Markedly hyperemic. Actual number and proportion of the polymorphonuclear elements apparently increased. Fat cells decreased. Numerous karyokinetic figures in the myelocytes, the latter being increased.

TABLE 6

DIFFERENTIAL LEUKOCYTIC COUNTS DURING HYPERLEUKOCYTOSIS PRODUCED BY INTRAVENOUS INJECTION OF BACTERIA *

Source of Blood and Time of Count		Total Count	Differential Count							
			Polymorphonuclears		Small Lymphocytes		Large Lymphocytes		Mast Cells	
			No.	%	No.	%	No.	%	No.	%
Left ear vein 2 minutes before injection of 60 million dead typhoid bacilli		9000	1350	15	6750	75	1260	14	90	1
Left ear vein 11 hr. after 1st injection and 5 min. before 2nd		8700	7221	83	1305	15	87	1	87	1
Left ear vein 13 hr. after 2nd injection of 60 million dead typhoid bacilli		24000	18960	79	3840	16	480	2	720	3
14 hr. after 2nd injection	Bone marrow	19600	14700	75	4704	24	196	1	0	0
	Superior mesenteric artery	16950	11357	67	4237	25	1017	6	339	2
	Superior mesenteric vein	19000	12160	64	5690	31	380	2	570	3
	Splenic artery	16750	10720	64	5360	32	335	2	335	2
	Splenic vein	91600	52212	57	35724	39	3664	4	0	0
	Splenic pulp	73000	26280	36	37960	52	8760	12	0	0
	Portal vein	16350	10628	65	5395	33	0	0	327	2
	Liver pulp	23100	14784	64	7623	33	462	2	231	1
	Hepatic vein	12950	8288	64	4144	32	259	2	259	2
	Lung pulp	30800	19404	63	8932	29	924	3	1540	5
	Right ventricle	23200	17632	76	4872	21	464	2	232	1
	Left ventricle	18000	7560	42	9180	51	540	3	720	4

* Rabbit 35, Table 5.

SUMMARY

In the blood of normal rabbits the leukocytes varied from 3300 to 14,650 per c.mm., with an average of about 9000. Normally, there were fewer per c.mm. in the blood of the left ventricle than in that of

the right. The blood from the normal liver and lung parenchymata contained approximately the same number of leukocytes as the blood in the general circulation, while in that from the splenic parenchyma the number was several times greater.

Following intravenous injection of bacteria there regularly occurred within a few minutes, usually less than 10, a great reduction in the number of leukocytes in the general circulation, chiefly involving the polymorphonuclear cells. The decrease, which amounted to 11,000 cells per c.mm. in some instances, was not dependent on the quantity of bacteria injected. The lowest count recorded was 1250 leukocytes per c.mm. The blood from the bone marrow, liver, and splenic parenchyma contained a greatly increased number of leukocytes, especially the blood from the latter, in which the count overreached 180,000 in several instances. The increase of leukocytes in the blood from these sources consisted largely of polymorphonuclear elements. The blood from the lung parenchyma did not contain an increase either in the number or proportion of the polymorphonuclear leukocytes. Late during the leukopenia, blood from veins of the internal organs and from the left ventricle contained more leukocytes than did that from the corresponding arteries and right ventricle. Sections of liver, spleen, and bone marrow revealed a hyperemic condition, especially marked in the case of the spleen.

From 4 to 15 hours after intravenous injection of bacteria there occurred a great increase of leukocytes in the blood of the general circulation, consisting largely of polymorphonuclear cells. No direct connection was observed between the quantity of bacteria injected and the height of the hyperleukocytosis. Sections of lung, spleen, and liver showed marked hyperemia; of the bone marrow, hyperemia and increased leukoblastic activity.

DISCUSSION

While there is an agreement among investigators concerning the occurrence of leukopenia and hyperleukocytosis after intravenous injection of dead bacteria, still a number of points remain unsettled. That leukopenia occurs is attributed to a withdrawal of leukocytes from the general circulation into the capillaries of certain internal organs. The evidence supporting this contention consists only of the easily demonstrated fact that there is a great diminution of the number of leukocytes in the general circulation, and an increase in the lung,

liver, and spleen during this stage, associated with an edematous condition of the endothelial lining of the capillaries.

If leukopenia is due to a mechanical obstruction, 2 questions arise: First, does the blood in the capillaries of these organs contain an increased number of leukocytes? And second, why is the mechanical sifting-out process confined practically entirely to the polymorphonuclear leukocytes, as demonstrated in this study, wherein the small and large mononuclear leukocytes are shown to have remained practically normal in the blood of the general circulation throughout the leukopenia?

If the leukopenia is due to a mechanical obstruction resulting in a retention of the leukocytes within the capillaries of the internal organs, examination of fresh blood obtained from these capillaries by deep incision into the parenchyma should reveal an increase in the number of leukocytes over that of the general circulating blood and blood obtained from normal organs; that this is actually the case has been demonstrated in this study (Table 3), for counts of blood obtained from the spleen and liver parenchymata, revealed an enormous increase in the number of leukocytes, reaching in the case of the spleen 186,000 per c.mm. It has been claimed that the lung capillaries play the most prominent part in this retention, but the results from this study assign minor importance to this organ in this connection, counts from its blood revealing in most instances little if any increase, tho sections showed some hyperemia and a slight increase.

On the other hand, it is difficult to conceive of a mechanical narrowing of the capillaries which would retard only the polymorphonuclear leukocytes. It has been advanced that the small mononuclear cells because of their smallness are able to pass through the narrowed capillaries, but the advocates of the mechanical theory apparently have not considered the fact that the many thousand polymorphonuclear leukocytes retained in the capillaries would in themselves furnish a mechanical barrier sufficient to produce a noticeable decrease in the small mononuclear cells in the circulating blood. While the blood from the liver and lung parenchymata during leukopenia suffers in part the same drop in polymorphonuclear elements as the peripheral blood, that from the spleen does not; here the actual number of polymorphonuclear cells per c.mm. is greatly increased. The results here adduced show that during the leukopenia the blood in the capillaries of the spleen and to a considerably less degree of the liver and lung, contains not only an increased number of leukocytes, but also an increased proportion of

polymorphonuclear elements; and that the lung does not contain a great increase of these cells, as has been reported. On the other hand, the blood from all sources, that from the splenic parenchyma excepted, contains practically a normal number of mononuclear cells. The mechanical theory, therefore, does not adequately explain the phenomenon of leukopenia.

Goldscheider and Jacobs[2] explained it on the theory of a negative or repellent chemotaxis, according to which the introduction into the general circulation of a foreign protein repels the polymorphonuclear leukocytes, causing them to find refuge within the capillaries of the internal organs. Certain results in this study furnish reasonable grounds for questioning this explanation.

Smears of the liver and splenic pulp of rabbits, made immediately after the onset of leukopenia produced by injections of living streptococci and staphylococci, revealed numbers of the injected organisms. Blood obtained from the parenchymata of these organs at the same time contained a greatly increased number of leukocytes, especially of the polymorphonuclear type, while the circulating blood contained relatively few cells of this type.

Bull[8] has demonstrated that organisms injected intravenously into animals disappear from the blood almost completely within 10 minutes after injection, and that many of these organisms can be found in sections of the liver and spleen. It is interesting to note that this period of time corresponds practically with that of the appearance of the leukopenia as found in this study.

While a negative or repellant chemotaxis is theoretically possible and as such might explain the production of leukopenia, still there is some doubt in the opinion of some observers as to the actual occurrence of a negative chemotactic action on leukocytes.[9, 10]

The evidence adduced here leads to the conclusion that leukopenia, at least in many instances, is not the result of a negative chemotaxis, but rather of a positive chemotaxis, acting on the polymorphonuclear leukocytes, which are chiefly concerned with phagocytosis, attracting them to the spleen and liver, and perhaps other organs, in which the injected or infecting organisms, or their products, have been filtered from the circulating blood; and because the other types of leukocytes are not so vitally concerned in phagocytosis they remain practically in normal numbers in the general circulation.

[8] Jour. Exper. Med., 1915, 22, p. 475.
[9] Cited by Wells, Chemical Pathology, 1907, p. 211.
[10] Compt. rend. Soc. de biol., 1912, 72, p. 722.

Whether the leukopenia occurring in typhoid fever, malaria, and other diseases can be explained on these grounds is not determined, but the enlargement of the spleen in these diseases supports, in part, this conclusion.

The striking findings in the spleen during leukopenia, and to a less degree during leukocytosis, have suggested an investigation of the effect of intravenous injection of bacteria in splenectomized rabbits. A report of these results will be made later.

During the stage of hyperleukocytosis many of the polymorphonuclear leukocytes present in the spleen and other organs during leukopenia, are discharged into the general circulation, this being indicated by the high counts of these cells in blood from the splenic and other veins in some cases. In addition the new polymorphonuclear cells produced in the bone marrow are thrown out into the circulation, further augmenting the number and proportion of these cells.

No relation between the height of the leukocytosis and the quantity of bacteria injected could be detected. Goldscheider and Jacobs[2] claimed that the degree of hyperleukocytosis was dependent on the quantity of protein injected; Lassabliere and Richet,[10] injecting albumin and peptone intraperitoneally, found no relation between the amount injected and the height of the leukocytosis. It was observed in this study that some rabbits would respond to a given dose of bacteria with a higher leukocytosis than others. Probably other, as yet undetermined, factors play an important part in the degree of leukocystosis produced.

CONCLUSIONS

Within a few minutes following the intravenous injection of dead bacteria into rabbits there results a marked decrease in the number of leukocytes in the circulating blood, resulting in a condition of leukopenia.

The decrease in the number of leukocytes involves chiefly the polymorphonuclear elements, in some cases these cells practically disappearing from the circulating blood.

Blood obtained from the splenic and hepatic parenchymata during leukopenia contains an enormously increased number of leukocytes per c.mm., as well as an increased number and proportion of polymorphonuclear elements.

The small and large mononuclear leukocytes remain practically normal in number in the blood from all sources, the splenic parenchyma excepted, during both leukopenia and hyperleukocytosis.

The mast cells may be increased late in leukopenia, just before the onset of hyperleukocytosis.

Blood obtained from the lung parenchyma during leukopenia does not contain an increased number of leukocytes, tho sections during this stage show a slight hyperemia and increase of these cells.

Blood from the bone marrow during leukopenia contains a normal or increased proportion of polymorphonuclear elements, with usually a somewhat increased total number of leukocytes.

Living organisms injected intravenously may be found in the liver and spleen a few minutes after the injection, synchronously with the onset of leukopenia.

A theory of the causation of leukopenia based on a mechanical obstruction to the passage of the leukocytes in the small capillaries of the internal organs does not adequately explain the phenomenon.

A negative chemotactic influence does not adequately explain the production of leukopenia.

The finding, during leukopenia, of injected living bacteria in the spleen and liver, synchronously with a great increase of polymorphonuclear leukocytes in these organs, and a great decrease of these cells in the general circulation, suggests that leukopenia is best explained on the basis of a positive chemotaxis.

Hyperleukocytosis is the result of the discharge from the spleen, liver, and perhaps other organs, of the leukocytes accumulated therein during leukopenia, plus the new cells produced in the bone marrow.

During hyperleukocytosis the blood from all sources in the body shows a great increase in the number and proportion of the polymorphonuclear leukocytes.